

Exposure to Ginger Root Oil Induces Male-Like Courtship Behavior in Female Mediterranean Fruit Flies (Diptera: Tephritidae)

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Abstract. Earlier observations revealed that females of *Ceratitis capitata* (Wied.) adopted a male-like, calling posture when exposed to the male attractant a-copaene. The present study expanded on this finding and, using ginger root oil (GRO, which contains a-copaene) quantified 1) the incidence of male-like wing movements as well as calling behavior for immature and mature females, 2) the effect of prior mating and different exposure regimes to GRO on the display of male-like courtship behaviors, and 3) the effect of exposure to GRO on female mating propensity. GRO's influence varied with female age and mating status. Immature females exposed to the chemical failed to exhibit any male-like courtship, and the incidence of male behaviors among treated females was much lower for mated than virgin females of similar age. GRO's effect also varied with the duration and timing of exposure and declined greatly after prolonged (2 h) exposure or removal of the GRO source altogether. Exposure to GRO, while acting to "masculinize" females, had no lasting effect on female receptivity, and treated and control females had similar mating frequencies in tests conducted 1 h or 2 d after exposure to the GRO source.

Introduction

The genetic basis of sexual behavior has been studied extensively in *Drosophila*, particularly in *D. melanogaster* Meigen (Yamamoto et al. 1997). Although most of this work has centered on males, observations of mutant females likewise reveal the importance of particular genes for the expression of sexual behavior. For example, *tra-2^{tsl}* mutant females of *D. melanogaster* exhibit appropriate behavior when raised under cool temperatures but exhibit male-like behavior if raised at high temperatures (Belote and Baker 1987; see also Cook 1975). Mutations may also affect receptivity in female insects. Females of *D. melanogaster* that carry the *spinster* mutant, for example, persistently reject males and display a much lower mating propensity than wild-type females (Hall 1994; see also Bellen and Kiger 1987).

Extrinsic factors are also known to influence the expression of sexual behavior in female insects. Diverse environmental factors, and particularly exposure to male sexual signals (Ewing 1964, Kyriacou and Hall 1982, Schein and Galun 1984) and adult diet (Lee 1955, Barton Browne et al. 1976, Schatral 1993), may strongly influence female receptivity (Ringo 1996). In contrast, there are, to my knowledge, only two published studies that document extrinsic control of the display of male-like sexual behavior by female insects. [Although not a natural environmental agent, juvenile hormone or analogues, when applied topically, have been found to both increase female receptivity (Ringo and Pratt 1978, Gadenne 1993, Yin et al. 1999) and induce male-like sexual behavior in females (Ringo and Pratt 1978).] Age-related expression of male-like courtship behavior has been noted for females in two species of Diptera. Working with a Hawaiian *Drosophila*, Ringo (1978) found that old, virgin females displayed three behaviors typical of lekking males: abdomen dragging, curl-

ing, and jousting. Similarly, Arita and Kaneshiro (1983) reported that old, virgin females of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) engaged in “pseudomale” behavior by assuming a pheromone-calling posture and making wing movements characteristic of courting males (see below).

Two studies have demonstrated a direct influence of an extrinsic factor on the display of male-like behavior by female insects. For females of *D. grimshawi* Oldenburg, Ringo (1978) showed that the age-dependent tendency to express male lek behavior was enhanced when females were exposed to the male sex pheromone. More recently, Nishida et al. (2000) provided experimental evidence that *C. capitata* females adopted a male-like, calling posture when exposed to the male attractant a-copaene, a sesquiterpene hydrocarbon found in a variety of host plants of the Mediterranean fruit fly (Nishida et al. 2000). Female response to this chemical was age-dependent: approximately 25% of mature females displayed male-like, calling behavior but only about 5% of immature females did so. Also, mounting attempts were observed occasionally among mature females exposed to a-copaene but never among immature females.

In studying the effects of ginger root oil (GRO), which contains a-copaene, on the mating performance of male Mediterranean fruit flies (Shelly 2001), I similarly observed females displaying male-like courtship when exposed to GRO. The purpose of the present study was to expand on the work of Nishida et al. (2000) and describe 1) the incidence of male-like wing movements as well as calling behavior for immature and mature *C. capitata* females, 2) the effect of prior mating and different exposure regimes on the display of male-like courtship behaviors, and 3) the effect of GRO exposure on female mating propensity. Data on female and male attraction to GRO as well as male response to GRO exposure will be considered in separate papers.

Knowledge of male courtship is essential for understanding the induction of male-like behaviors in females. The basic sequence of the courtship behavior of *C. capitata* males has been described by several authors (e.g., Feron 1962; Arita and Kaneshiro 1986, 1989; Liimatainen et al. 1997), and the following summary derives from these accounts. Males defend individual leaves on host and non-host trees as mating territories. While perching, males display “calling” behavior in which they hold the wings away from the body, curve the abdomen upward, and evert the rectal epithelium (producing a bubble-like structure), thereby dispersing a pheromone attractive to females. Upon detecting a female, the male bends his abdomen ventrally (with the rectal epithelium still everted) and begins to vibrate his wings (an action termed wing vibration). Following approach by the female to within 2–5 mm, the male usually initiate a different type of wing movement (superimposed on continuing wing vibration) in which the wings are moved rhythmically forward and then back (an action termed wing buzzing). During wing buzzing, the male also moves his head back-and-forth (an action termed head rocking). Although males rarely display wing buzzing without first exhibiting wing vibration, the progression from vibration-to-buzzing is not strictly followed, and males may continue wing vibration (without displaying wing buzzing at all) or may alternate bouts of wing vibration and buzzing. If the female remains stationary throughout these close-range displays, the male suddenly jumps on top of the female and attempts to mount and copulate.

Materials and Methods

Study animals. With the exception of one experiment, the flies used in this study were derived from a laboratory colony started with 300–400 adults reared from fruits of coffee (*Coffea arabica* L.) collected near Haleiwa, Oahu (females from this strain were termed wild-like). Adults were held in screen cages and provided with a sugar-yeast hydrolysate

mixture (3:1 by volume), water, and an oviposition substrate (perforated plastic vials containing small sponges soaked in lemon juice). Eggs were placed on standard larval medium (Tanaka et al. 1969) in plastic containers over vermiculite for pupation. Adults used in the present study were separated by sex within 1–2 d of eclosion, well before reaching sexual maturity at 6–9 d of age, and kept in screen-covered plastic buckets (5 liters volume; 100–125 flies per bucket) with ample food and water. The flies were maintained at 20–24 °C and 65–85% RH and received both natural and artificial light with a photoperiod of 12:12 h (L:D). When observed, the wild-like flies were eight generations removed from the wild. In addition, wild flies were used in one set of trials to confirm that the behavior of the wild-like females was not a laboratory artifact. The wild females observed were reared from fruits of the loquat (*Eriobotrya japonica* (Thunb.)) collected on Kula, Maui, and were maintained following the protocol described above.

Performance of male-like courtship behavior. The influence of GRO on female behavior was studied in six experiments, and the same basic protocol for exposing females was followed throughout. I applied 20 ml of GRO to a small disc of filter paper using a microcapillary pipette. The oil (obtained from Citrus and Allied Essences, Ltd., Lake Success, NY) contained a-copaene in low concentration (0.4%, T.W. Phillips, personal communication), with the (+) enantiomer predominating (81%). The disc was placed on the bottom of a transparent, plastic drinking cup (400 ml volume), and six females were immediately placed in the cup using an aspirator. Nylon screening was then placed over the top of the cup. In all experiments, behavioral observations were made for four cups simultaneously every 2 min over a 30 min interval ($n = 16$ total checks). During the checks, the cups were scanned individually for 3–5 s, and the incidence of particular behaviors was recorded (see below). Observations were made between 0900–1200 hrs over all experiments under the laboratory conditions described above.

The six experiments conducted differed with respect to the age (11–13 d old [mature] or 1 d old [immature]), mating status (virgin or mated), and strain (wild-like or wild) of the females tested, the duration of the exposure period, and the presence/absence of GRO during observations. Using wild-like females, I made observations of: 1) mature, virgin females exposed to GRO for 10 min prior to and during observations; 2) immature, virgin females exposed to GRO for 10 min prior to and during observations; 3) mature, mated (2 d before observations) females exposed to GRO for 10 min prior to and during observations; and 4) mature, virgin females exposed to GRO for 2 h prior to and during observations. In experiments 1–4, the GRO-containing disks were left in the cups during observations, but I also observed (5) mature, virgin females exposed to GRO for 30 min and then isolated from the GRO-containing disk for 1 h prior to observations. To confirm that the male-like behavior observed was not a consequence of laboratory rearing, a sixth experiment was performed that was identical to experiment 1 except that wild females were used (13–14 d old).

For all experiments, observations were also made of control females (identical in age, mating status, and strain to the corresponding group of treated females) using the protocol described above except that no GRO-containing disks were placed in the observation cups. Control females were maintained and observed in a separate room isolated from the odor of GRO. Observations of control females were made simultaneously with those of treated females. Twelve cups of treated females and 12 cups of control females were observed for each experiment.

During the periodic behavioral checks, we recorded the incidence of: 1) calling behavior (wings held laterally, abdomen turned upward; no bubble-like structure was apparent during female calling); 2) undirected wing vibration (wings vibrated by walking or stationary females not in close, face-to-face courtship position with other female); 3) courtship (two stationary females 2–5 mm apart facing one another with at least one individual exhibiting

wing vibration or wing buzzing); 4) oviposition behavior (abdomen arched upward, ovipositor extended downward and probing surface); and 5) mounting attempts (one female jumps on the back of another). As also noted by Arita and Kaneshiro (1983) and Nishida et al. (2000), the calling posture and wing actions displayed by females were indistinguishable from those of males (see Nishida et al. 2000 for a photograph of a calling *C. capitata* female). Aggressive interactions, chiefly one female lunging briefly at another, were observed only infrequently, and data on their occurrence are not reported herein.

Mating propensity. Because exposure to GRO induced male-like courtship in certain circumstances, I investigated the possibility that GRO exposure affected female mating propensity. The mating frequencies of GRO-exposed and non-exposed wild-like females were compared in two experiments. In the first, the treated females were exposed for 1 h (0630–0730 hrs) immediately before the mating trial, and in the second, the treated females were exposed to ginger root oil for 6 h (0700–1300 hrs) 2 d before the mating trial. In both cases, the exposure protocol was the same as that outlined above, except 25 females were placed in each of the cups. In both experiments, females were 9–10 d old when exposed.

Mating tests were conducted at the Agricultural Experiment Station of the University of Hawaii, Waimanalo, Oahu. Groups of 75 treated and 75 control (non-exposed) females and 150 males (8–12 d old) were released between 0730–0830 hrs in field-cages (height: 2.5 m, diameter 3.0 m) that contained a single rooted guava tree (*Psidium guajava* L.). For a given trial, we marked either treated or control females, alternating the marked group between successive trials. Treated females were marked 1 d prior to exposure, and control females were marked 1 d prior to the mating test. Females were marked by cooling them for several minutes and placing a dot of enamel paint on the thorax. This procedure had no obvious adverse effects, and females resumed normal activities within minutes of handling. The cages were monitored continuously for 4 h, mating pairs were collected in vials, and the females identified. Nine replicates were conducted for each experiment.

Statistical analyses. The Mann-Whitney test (test statistic T) was used to compare the incidence of calling behavior, undirected wing vibration, courtship, and oviposition behavior between treated and control females for a given experiment and the number of matings involving control versus treated females in the field cages. For treated females, the incidence of these behaviors was compared among the different experiments with a Kruskal-Wallis test (test statistic H), and if significant variation was detected, a Tukey test was used to make pairwise comparisons. Analyses were completed using SigmaStat Statistical Software.

Results

Performance of male-like courtship behavior. The influence of GRO on female behavior was age-dependent, and immature females (treated or control) displayed no male-like or ovipositional behavior (experiment 2, Table 1). Consequently, data from this experiment were excluded in the subsequent analyses.

Treated females displayed higher frequencies of calling, undirected wing vibration, courtship, and oviposition than control females in all experiments (Table 1; $P < 0.01$ in all cases). Control females did not display calling behavior in any experiment but exhibited all other behaviors at very low frequencies in all experiments.

For treated females, significant variation was detected among experiments in the frequency of calling behavior ($H = 47.0$, $df = 4$, $P < 0.001$) and courtship ($H = 42.1$, $df = 4$, $P < 0.001$). A similar pattern of inter-treatment variability was evident for these two behaviors: mature, virgin females from wild-like and wild strains that were observed 10 min after the GRO was introduced (experiments 1 and 6, respectively) had high, and statistically indistinguishable, frequencies of both calling and courtship relative to treated females in all

Table 1. Incidence of calling behavior, undirected wing vibration, courtship, and oviposition by *C. capitata* females during the six experiments of the study¹.

	Calling	Undirected wing vibration	Courtship	Oviposition
Experiment 1				
Treated	19.8 ^a (2.3)	2.5 ^a (0.4)	11.6 ^a (1.5)	6.7 (1.9)
Control	0	0.1 (0.1)	0.6 (0.2)	1.8 (0.4)
Experiment 2				
Treated	0	0	0	0
Control	0	0	0	0
Experiment 3				
Treated	5.0 ^b (1.3)	1.4 ^{ab} (0.3)	2.3 ^b (0.4)	1.6 (0.5)
Control	0	0.1 (0.1)	0.2 (0.1)	1.9 (0.8)
Experiment 4				
Treated	4.2 ^b (0.9)	1.0 ^{ab} (0.2)	2.4 ^b (1.0)	2.7 (0.4)
Control	0	0.1 (0.1)	0.2 (0.1)	1.7 (0.4)
Experiment 5				
Treated	0.3 ^b (0.1)	0.3 ^b (0.1)	0.5 ^b (0.2)	2.3 (0.8)
Control	0	0.1 (0.1)	0.1 (0.1)	2.1 (0.5)
Experiment 6				
Treated	24.0 ^a (1.7)	1.8 ^a (0.5)	8.3 ^a (1.3)	2.8 (0.8)
Control	0	0.2 (0.1)	0.5 (0.3)	0.1 (0.1)

¹For all experiments, values represent means (SE) per replicate (female groups; n = 12 per experiment). Courtship values represent pairs of individuals; values for all other behaviors represent individual females. For a given behavior, values for treated females followed by a shared letter were not significantly different ($P = 0.05$; Tukey test); data from experiment 2 were excluded from the analyses.

Experimental treatments:

1. wild-like, mature, virgin females observed 10 min after GRO introduced.
2. wild-like, immature, virgin females observed 10 min after GRO introduced.
3. wild-like, mature, mated females observed 10 min after GRO introduced.
4. wild-like, mature, virgin females observed 2 h after GRO introduced.
5. wild-like, mature, virgin females observed 1 h after GRO removed.
6. wild, mature, virgin females observed 10 min after GRO introduced.

other experiments (Table 1). Mating (experiment 3), prolonged exposure (experiment 4), and removal of the GRO source (experiment 5) resulted in a dramatic decrease in behavior frequencies, which did not differ significantly among treated females in these experiments. Mounting was only rarely observed but displayed a similar pattern of occurrence: seven and five mountings were recorded over all replicates of experiments 1 and 6, respectively, and

only two mountings were observed over all replicates of experiments 3, 4, and 5 combined.

The frequency of undirected wing vibration also differed significantly among treatment groups ($H = 17.7$, $df = 4$, $P < 0.05$). The pattern of occurrence of this behavior was similar to that observed for calling and courtship (Table 1). However, the frequency of this behavior was low over all experiments, and fewer significant differences were detected between treatment groups. Variation in the incidence of oviposition behavior was not significantly different among the treated females in the different treatments ($H = 4.9$, $P > 0.05$).

Mating propensity. Exposure to ginger root oil had no apparent effect on female mating propensity in either of the two experiments conducted. When exposed on the day of testing, treated females participated in an average of 21.6 matings per replicate (range: 9-36) compared to 24.5 (range: 8-43) for control females ($T = 91.0$; $n_1 = n_2 = 9$; $P > 0.05$).

In the second experiment, where treated females were exposed 2 d before the mating trial, 22.0 treated (range: 10-35) and 23.8 control (range: 9-37) females mated, on average, per replicate ($T = 94.0$; $n_1 = n_2 = 9$; $P > 0.05$).

Discussion

The data presented herein allow the following four conclusions. (1) GRO-induced expression of male courtship behavior by *C. capitata* females was not an artifact of laboratory rearing, because wild females also displayed male behaviors in the presence of GRO. (2) GRO's influence varied with female age and mating status. Immature females exposed to the chemical failed to exhibit any male-like courtship, and the incidence of male behaviors among treated females was much lower for mated than virgin females of similar age. (3) GRO's effect varied with the duration and timing of exposure and declined greatly after prolonged (2 h) exposure or removal of the GRO source altogether. (4) Exposure to GRO, while acting to "masculinize" females, had no lasting effect on female receptivity, and treated and control females had similar mating frequencies in tests conducted 1 h or 2 d after exposure to the GRO source.

Given Arita and Kaneshiro's (1983) observation of age-related expression of male behaviors by *C. capitata* females, it appears that GRO simply advances the expression of male-like courtship among mature females. That is, among mature, virgin females GRO triggers the immediate display of male-like behaviors that are usually (if at all) expressed only relatively late in life, i.e., after the typical age of mating. This same explanation may account for heightened female display of male lek behavior in the presence of male odor reported for *D. grimshawi* (Ringo 1978). In contrast, juvenile hormone has a delayed action, and female display of male behaviors was not evident for several weeks following topical application of the hormone (Ringo and Pratt 1978).

Regardless of the mechanism, the present findings (along with those of Arita and Kaneshiro 1983 and Nishida et al. 2000) reveal that the neural 'machinery' required for the production of male courtship behavior are present in females. Ringo (1978) has proposed that the expression of male courtship behavior in female *Drosophila* reflects the recent phylogenetic origin and ongoing evolution of this behavior. Whether this explanation accounts for the incomplete behavioral dimorphism evident in *C. capitata* remains unknown.

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